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TOXICITY OF PYRETHROID, CYPERMETHRIN ON NEUROSECRETORY ACTIVITY IN FRESHWATER SNAIL, *LYMNAEA ACUMINATA* (L)

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Abstract

A detailed study on the morphology of nervous system, neurosecretory cells and the seasonal variations in the neuro secretory cells along with effect of sublethal concentration of cypermethrin in a freshwater snail, Lymnaea acuminata has been carried out. The nervous system consists of several paired as well as unpaired ganglia forming a ring, like a pair of cerebral, pleural, pedal, parietal ganglia and a single visceral ganglia. Two types of neurosecretory cells, A and B, were identified by using Mallory's triple staining method. A cells were found to be pyriform in shape with long axon while B cells were round without axon. Seasonal variations in neurosecretory cells were observed in cerebral ganglia along with the exposure to sublethal concentration of cypermethrin for 24 and 96 hours exposure span. It was found that the number of neurosecretory cells (A and B) decrease progressively after 24 and 96 hours exposure period.

Key words: *Lymnaea acuminata, Cypermethrin toxicity, neurosecretion*

Introduction:

The presence of prominent inclusions suggestive of secretory activity in the neurons of gastropods, has attracted the attention of a number of investigators (Scharrer and Scharrer, 1954; Chou, 1957 ; Amorso *et al.*, 1964 ; Ladislay, 1966). Gryzcki (1951) studied the structure of neurosecretory material in the ganglion cells *of Planorbis* and *Paludina.* Chau (1957) observed the cytoplasmic inclusion of the neurons *of Helix aspersa* and *Lymnaea stagnalis.* Neuroendocrinology is usually regarded as the study of mutual

interactions between the two integrative systems, *viz.*, the nervous system and endocrine system of metazoa (Scharrer, 1967). The study of the neuroendocrinology of invertebrates is, to a considerable extent, the study of neurosecretion in these animals. The effect of different environmental parameters and transquilizers on the neuroendocrine regulation was studied in the slug, *L. alte* (Kulkarni, 1970). Hazari (1983) has studied in detail the activity of neurosecretory cells in the snail, *Cerastus moussonianus.* Jawalikar (1989) and Shinde (1991) have described in detail the anatomy and mapping of CNS and identified different Neurosecretory cells in *L. alte* and *Indoplanorbis exustus* respectively.

A large number of substances and pesticides have been successfully proved to have molluscicidal effects and being used for the control of molluscan pests in different parts of the world, but very few reports are available on molluscs in relation to the effects on the neurosecretion. Most of the work that has been done on the effects of pollutants on neurosecretion is dealt in vertebrates especially in fishes. Among invertebrates few reports are available on the effects of pesticides on neurosecretory activity in insects and molluscs. Organophosphate compounds kills animals by inhibiting colinesterase activity with consequent disruption of nervous activity caused by accumulation of acetylcholine at the nerve endings (Fukuto 1971; Gupta 1971 and Fest and Schmidt 1973) thereby interfering with nerve conduction mechanism.

Few workers also have studied the pesticidal effect on different species of molluscs. Mane *et al.* (1979) have studied the effect of pesticides and narcotants on bivalve molluscs, *Katelysia opima* and *Donax cuneatus.* Bodhankar (1984) has studied the effect of pesticides exposure on neurosecretory activity of *Laevicaulis alte* by using pesticides, viz., Malathion, Hygro, Sevimol, Thiodon and Copper sulphate. Muley (1985) has worked out the effects of pollutants on different freshwater molluscs from Godavari river.

Since no work has been carried out on pulmonates especially on the freshwter snail, *L. acuminata,* of the neuroendocrine activity in relation to emphasize the changes in the number of neurosecretory cells of the central nervous system of *L. acuminata.* The present investigation deals with the study of a Pyrethroid, Cypermethrin in relation to the neurosecretory activity in *L. acuminata.*

MATERIAL AND METHODS

The freshwater pulmonate snail, *L. acuminata* were collected from Kham river and local ponds near Aurangabad. The snails were brought to the laboratory and cleaned and placed in troughs containing sufficient dechlorinated tap water. The snails were fed once in a day with plant vegetation like Hydrilla and algal material like Spirogyra. The water from the troughs was changed twice in a day.

For the experimental purpose only active and healthy snails of approximate equal size and weight were selected. A group of 25 snails were subjected to sub lethal treatment of Cypermethrion for 1 and 4 days. A control group of snails was also maintained simultaneously. After completion of exposure period, that is 1, 4 days and control, the snails were dissected out and central nervous system was separated and fixed in Bouin's fluid, followed by microtechnique to prepare 5 – 7 u thickness sections. The sections were stained with Mallory triple stain (Mallory, 1944). The monthly observations on the activity of the neurosecreteory cells were made to know the changes in neurosecretion. The observations were made at light microscopic level.

OBSERVATIONS AND RESULTS

The central nerrous system of *Lymnaea acuminata* is in the form of a ring with a pair of cerebral, buccal, pleural, pedal, parietal and visceral gnaglia. All these ganglia are held together with their commissures, with the ring formation due to the presence of connective between these ganglia . The ring is situated posteriorly to the buccal mass. From the buccal mass arises oesophagus which passes backwards through the ring of ganglia. Nerves arise from the ganglia and reach to their respective target organs.

In *L. acuminata,* dorsal body is closely associated with the central nervous system, situated in close proximity, mid-dorsally to cerebral ganglia. Generally a single body is observed and the shape of the body appears to be like a kidney or it is oblong. The shape and size of the body depends on the functional status of it. Internally the body bears continuity with cerebral ganglia through intercerebral commissure.

The neurosecretory cells are quite distinct from the normal neural cells. These neurosecretory cells are bigger with conspicuous nuclei and large amount of cytoplasm. Their perikarya and axons, in A, cells are loaded with fine particles, stained distinctly. These staining peculiarities

are characteristic features of neurosecretory cells and thus differentiates them from the normal neuronal cells.

On the basis of their morphological features like size, shape, vacuolization, stainability etc., the neurosecretory cells of *L. acuminata* could be classified into two chief types, (1) A cells / I cells (axonic) and (2) B cells / II cells (non-axonic). These cells were observed in cerebral, buccal, pleural, parietal and visceral ganglia and more generally arranged in cerebral, buccal, pleural, parietal and visceral ganglia leaving in the centre a lumen, neuropile. The extreme periphery of ganglia is suposed to be the neurohaemal region as being observed to be vacuolarized . The number, size and shape of these cells may vary from ganglion to ganglion. The characteristics of these cells have given in table 1.

Cell type A (Fig. 1):

These cells are pyriform in shape and have long axons. The length of the cell body ranging from 25-55 u. The size of cell body varies considerably and depends upon the functioned status of the cell. The nuclei may be ovoid or pyriform and their diameter ranges from 6-10 u. Their size and number is comparatively smaller than B cells. They are located with B cells and generally lie towards the peripheral region of the ganglia. The nucleus generally bears single nucleolus. The cytoplasmic portion of the cell consists a colloidal neurosecretory material stained deep blue with Mallory triple staining and contains few vacuolar spaces. The neurosecretory material appears to be within axonic processes suggesting the usage of axonal passage for the transportation of neurosecretory material elaborated by these cells.

Cell type B (Fig. 1) :

These types of cells are oval or round, large than A cells and range from 50-95 *u* in length. The cells lack any cellular processes (axon). The nuclear diameter is also large than A cells and range from 20-25 u. The nucleus occupies the major portion of the cell body. The number of B cells is more when compared to A cells and the cells are generally located in subperipheral region of the ganglion. The cytoplasmic portion includes, granular neurosecretory material stained red with Mallory triple stain. The number and size of the cells may vary from ganglion to ganglion and the number containing neurosecretory material (NSM) was increased particularly during breeding season.

Changes in neurosecretory cells and reproductive cycle :

The number of A and B cells from the cerebral ganglia of *L acuminata* has been surveyed from August 2010 to July 2011 and that the statistics are presented in Table 2. From the data, it is clear that the number of B cells containing NSM was maximum from June to September. The number decreased gradually in the next period up to February. From March a progressive increase in the number of B cells was observed.

After 1 day exposure to cypermethrin, a considerable decrease in number of 'B' cell where as on 4 days exposure to cypermethrin significant decrease was observed

'A' cells also showed seasonal variations but these were not remarkable. However, the number of 'A' cells were found to be increased in the months of November to January and depleted during breeding season (July to October). The decrease or loss in number of 'A' cells during breeding season is replaced in the months of November to January and the period of these months is designated as recovery period / post-breeding season.

During the period of June to September, *i.e.*, breeding period the activity of 'B' cells was maximum. The number was increased to its maximum extent; the nuclear diameter was also increased. The neurosecretory material in the cytoplasm was also more giving maximum staining intensity. At the same time number of 'A' cells containing NSM were decreased and they devoid of stainable neurosecretory material.

During recovery period, 'A' cells were found to be increased, in their number, size and stainability. The length of axonic filament was also increased and stained nicely suggesting that the nuerosecretory material flowed out through them. The number of 'A' cells during end of recovery period was found to be negligible and lowest in the month of May. At the end of recovery period, the number of 'B' cells increased to some extent and the cytoplasm appeared to be filled with stainable material.

During later three months (March, April and May), both types of cells were found to be less in number with reduced activity. With the beginning of next breeding cycle, the synthesis and number of both the cells showed increased activity from June. Whereas 'B' cells progresively increased and more neurosecretory material appeared in their cytoplasm than A cells.

DISCUSSION

Molluscan pharmacology has collected considerable interest in recent years. One of the concerns is that chemical transmission within the nervous system and neuromuscular system of molluscs and the concern is toxic materials elaborated by molluscs. Despite its relative simplicity, the molluscan brain has basic structural and functional features which are similar to those found in vertebrates brain (Endean, 1972). However, studies of molluscan nervous system have been facilitated by the presence giant neurons. These giant neurons can readily be identified because of their size (150-500 µ in diameter) and their positions with respect to other neurons. Their superficial location renders them amenable to investigation involving microelectrodes and iontophoretic application of drugs. It is to be expected that studies of the identifiable neurons present in molluscs will lead to elucidation of the roles of several chemical compounds which are believed to function as neurotransmitters and will increase our knowledge of the chemical heterogeneity of the brain. In this respect, it should be noted that many pharmacological agents which modify neuronal activity in vertebrates are functional within molluscan nervous system (Endean, 1972).

Few literatures are available on relationship between neuro-secretion and reproduction in molluscs. Gabe (1954) first reported cyclic neurosecretory activity correlated with the reproductive cycle in a number of ophisthobranchs and pulmonates, Pelluet and Lane (1961) have observed that the neurosecretory activity is correlated with hermaphrodite gland activity in slugs, *Arion ater* and *A. subfiiscus.*

Neurosecretory cells in the cerebral ganglia of the snail, *Lymnaea acuminata* show distinct staining activity with Mallory triple staining during its annual cycle. The two types of neurosecretory cells (NSCs), 'A' and 'B' were seen and found to be scattered in all ganglia. In cerebral ganglia number of B cells increased during breeding season (i.e. from July to September). The numbers of 'B' cells were decreased from November onwards. Somewhat reverse condition was happened with number of 'A' cells. Simpson *et al.* (1966) have also mapped seasonal variation in the number of fuchsinophilic cells in various ganglia of the central nervous system of *Heliosoma tenue.* Joosse (1964) also observed the annual cyclic activity in neurosecretory cells of the cerebral ganglia and dorsal bodies of the snail, *L. stagnalis.* In *Lymnaea acuminala* maturation of gonads is related with neurosecretion. Neurosecretory material was found to be accumulated during the growth of the gonads (showig more GSI), whereas it was found to be decreased after breeding. Thus, it is clear that in this snail, neurosecretory activity and the reproductive

activity are correlated to each other. Similar results have also been reported in the slug, *Laevicaulis alte* (Nagabhushnum and Kulkarni, 1971), in the aquatic snail, *Indoplanorbis exustus* (Shinde, 1991) and *Thiara lineata* (Ahirrao and Khedkar 2012) .

Effect of Pesticide toxicity:

The toxicity of a substance when tested on an organism or when applied in the environment depends not only on the concentrations of the substance, but also on the particular chemical and physical conditions under which it is tested (Eisler, 1970). Various animals exhibit varying degrees of susceptibility to pesticides. The factors affecting the toxicity of a particular pesticide are animal weight (Pickering *et al.,* 1962), its development stage (Kamaldeep and Toor, 1977), time of exposure and temperature (Macek *et al.,* 1969), pH and hardness of water (Handerson *et al.,* 1960). Most of the evidences are available on the toxicity. The effect of reserpine and chlorpromazine on the neurosecretory cells of saline treated bivalve *Indonaia caeruleus* have been studied by Hanumante *et al.* (1978) and they showed that after saline treatment A and B cells of cerebral and visceral ganglia showed significant increase in cell area, nuclear diameter and decrease in neurosecretory material while after administration of reserpine and chlorpromazine complete inhibition of these changes were observed.

It is a well known fact that the neurosecretory centres control the physiological processes like reproduction and exposure of animals to pesticides interfere with the normal function process and ultimately create an imbalance in the normal system. Utkar (1982) has found the toxic effect of copper sulphate on the neurosecretion and has reported a decrease in cell number cell and nuclear area, depletion of NSM in the neurosecretory cells of the freshwater snail, *Vtviparus bengalensis.* He also reported that effect of biogenic amines (reserpine and esperpine) on nueroseceretory cells and observed that the size of both neurosecretory cells (A and B) was reduced. Bodhankar (1984) observed that there was decrease in NSM in both A and B type of cells, number of cell types, and cell and nuclear areas of these cells of the slug, *Laevicaulis alte* on exposure of five different molluscicides, *viz.,* Malathion, Hygro, Thioden, Sevimol and Copper sulphate. Bhatlawande (1989) has reported similar phenomenon in the snail, *Cerastus moussonianus.* Ahirrao and Khedkar(2012) while working on a freshwater snail, *Thiara lineata* found that the Sevin have degenerative effects on the neurosecretory cells accompanied with depletion in neurosecretory material, decreased in nuclear diameter

and cell number of the neurosecretory cells. In the present investigation on exposure of cypermethrin, it was oberved that there was an acute cellular degeneration, vacuolization and pronounced decrease the cell number in cerebral ganglia *of Lymnaea acuminata.* These results are in agreement with the result of Utkar (1982), Bodhankar (1984), Bhatlawande (1989) and Ahirrao and Khedkar (2012).

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Table1: Morphological properties of neurosecrtory cells of cerebral ganglion of *L. acuminata.*

Particulars	**A cells**	**B cells**
Shape of the cell body	Pyriform	Oval
Size of the cell body	Smaller than B cells (25-55 µ)	Larger than A cells (50-95 µ)
Nucleus	Ovoid or Pyriform	Oval
Nuclear diameter	6-10 µ, increase during post breeding season	20-25 µ increases during breeding season
Axonic process	Present	Absent
Vacuole	Present	Absent
The cytoplasmic granule (NSM) stained with MTS	Deep blue	Red
Nature of neuro-secretory material	Colloid	Granular
Glycogen	Absent	Absent

Proteins	Present	Present
Fats	Present	Present

Table 2: Variations in the number of stainable neurosecretory cells (NSCs) present is cerebral ganglia of *L. acuminata* from August 2010-July 2011 on exposure to Cypermethrin.

Year	No. of 'A' cells			No. of 'B' cells		
	Control	1 day	4 days	Control	1 day	4 days
2010 Aug.	11	10	10	43	42	38
Sept.	06	06	05	34	32	30
Oct.	06	05	05	23	21	20
Nov.	12	11	10	19	18	15
Dec.	19	17	15	13	12	11
2011 Jan.	13	11	09	09	08	07
Feb.	08	08	06	09	08	07
Mar.	07	07	06	12	10	09
Apr.	08	08	06	14	12	11

May	05	04	04	15	13	12
June	07	06	05	28	26	23
July	09	08	08	32	30	27

Figure 1:Variations in the number of "A" cells (NSCs) present is cerebral ganglia of L. acuminata from August 2010-July 2011 on exposure to Cypermethrin.

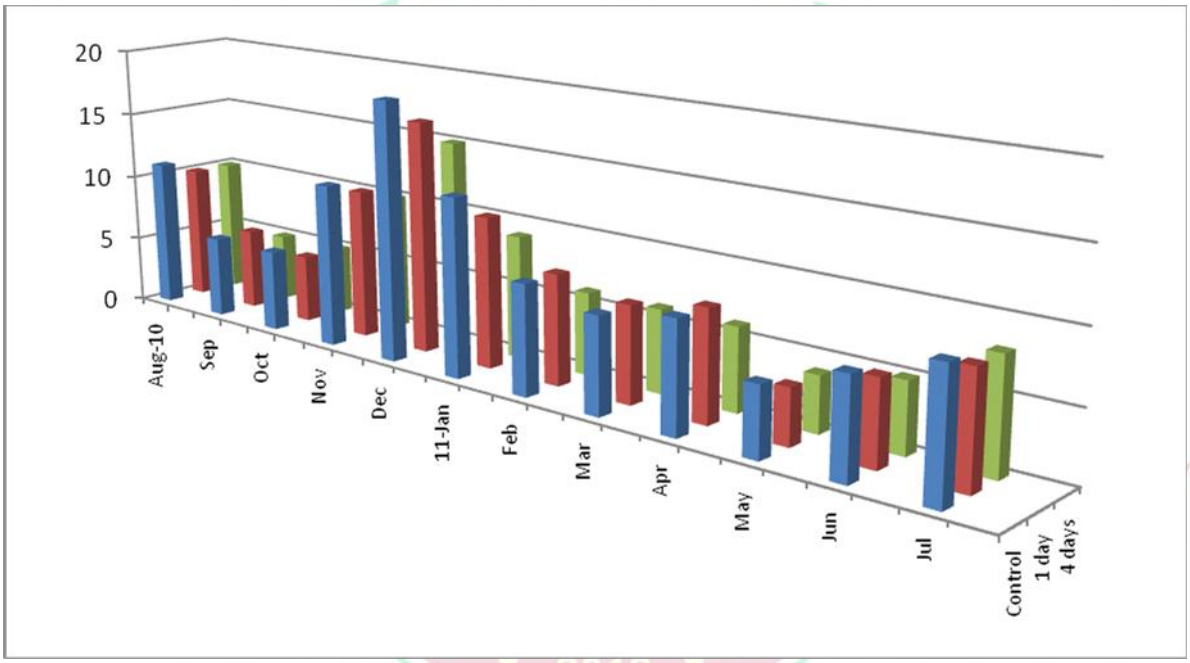

Figure 2:Variations in the number of "B" cells (NSCs) present is cerebral ganglia of L. acuminata from August 2010-July 2011 on exposure to Cypermethrin.